was extended to 5 minutes, a purple color indicating oxidation of the *p*-phenylenediamine hydrogen donor was noted in the three siblings with the low progress curves (Table 1). In the absence of an inflammatory response (a count of less than $9 \times 10^3$ white cells per cubic millimeter of venous blood), enzyme samples from controls failed to give any measurable color after long incubation in the absence of added hydrogen peroxide. This finding is interpreted to indicate the presence in the sample from the three siblings of increased amounts of endogenous peroxide, probably hydrogen peroxide, which is gradually reduced by the residual enzyme. It is proposed that demonstrable intracellular peroxide levels are a result of the peroxidase enzyme deficiency.

Acid phosphatase (*5*), catalase (*6*), and superoxide dismutase (*7*) were assayed in the leukocyte preparations; the activities of the three siblings did not differ from controls. Thus, the abnormality of peroxidase in the leukocyte preparations did not extend to an enzyme representative of the lysosomal hydrolases, to another hydrogen peroxide–consuming enzyme, or to an enzyme known to produce hydrogen peroxide. In azurophilic hypergranulation (*4*) in which the Giemsa stain at *p*H 6.5 was used, mild hypergranulation was found in both patients affected with the disease. No hypergranulation was observed in the unaffected sister. The deficiency in leukocyte peroxidase *p*-phenylenediamine thus could be used to correctly diagnose both patients with Kuf's disease independently of previous hypergranulation studies. However, the superiority of the peroxidase assay is shown by the detection of an abnormal enzyme in the apparent heterozygous unaffected sister (IV/11).

The peroxidase reaction studied is extremely rapid. In the normal progress curve (Fig. 1), the rate decreases after 30 seconds of incubation. If one takes the difference in readings between 10 and 30 seconds of incubation as an approximation of the true initial rate, then the samples from the two clinically affected siblings have initial rates of activity much below that of the lowest control and the sample from sibling IV/11, for which the progress curve falls below that of the lowest control, has, in fact, a normal initial rate. It is important, therefore, to examine the entire curve (10 to 100 seconds) as well as the initial rate. Thus, enzyme activity for patient IV/13 falls in the low normal range at 10 seconds, but formation of reduced substrate then neither continues at the normal rate nor reaches the full normal value. Therefore, by extending the assay time, the full extent of the peroxidase deficiency is revealed. The significance of the abnormal progress curve in sibling IV/11 is not apparent. Although she is beyond the age when other members of her family first developed symptoms of the disease, she might carry the trait. If she does, the progress curve determined from her leukocytes suggest that the enzyme abnormality may be in the stability of the protein rather than in initial catalytic activity.

Table 1. Oxidation of *p*-phenylenediamine in the absence of added hydrogen peroxide by white cell preparations. The reaction described in Fig. 1 was performed in the absence of added hydrogen peroxide and the absorbance at 485 nm was measured after 5 minutes of incubation.

| Enzyme source | White cell count (cell/mm$^3$) | Absorbance at 485 nm |
|---|---|---|
| Controls | $<9000$ | 0.000 |
| IV/11 | 6000 | .006 |
| IV/13 | 4900 | .024 |
| IV/15 | 6400 | .054 |

The use of a specific hydrogen donor (*p*-phenylenediamine) is stressed because when other donors are employed, the enzyme deficiency may not be demonstrated. Others have confirmed this observation (*8*). Porphyria (*9*) is the only dominantly inherited disease in which an enzyme abnormality has been detected. Our findings suggest an enzyme abnormality in another dominantly inherited disorder with chronic progressive degeneration of the nervous system. Further studies of this pedigree and of the nature of the enzyme abnormality should shed light on an additional mechanism for expression of the biochemical phenotype of dominantly inherited diseases and should enable precise biochemical detection of individuals bearing the trait.

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## Responses in Pavlovian Conditioning Studies

Wasserman (*1*) reported that in a cooled chamber, young chicks came to approach and peck a small disk whose illumination was repeatedly followed by heat lamp activation. One central conclusion he drew from these data was: "Approach and contact of conditioned stimuli does not depend on similar or compatible conditioned stimulus– and unconditioned stimulus–controlled responses. The chicks approached and pecked or snuggled with the lighted key even though the heat stimulus evoked none of these behaviors." This conclusion is important because it appears to be an exception to a generally accepted interpretation of previous studies using similar procedures (*2*): Behavior toward the conditioned stimulus has always been similar either to behavior elicited by the unconditioned stimulus or to behavior related to the motivational state associated with the unconditioned stimulus.

Thus, when food or water have been used as unconditioned stimuli in similar experiments, approach and contact of a lighted key (conditioned stimulus) could be considered part of normal eating or drinking behavior, redirected toward a new stimulus.

Observations of a broody hen with young chicks in my laboratory suggest, however, that approaching, pecking, and snuggling are part of the normal heat-seeking behavior of young chicks. I have kept a mother hen with small groups of 3- to 8-day-old chicks in a large observation cage (1.2 by 2.4 m) for classroom demonstrations. On many occasions I have observed that one or more of the chicks will approach the hen—which may be feeding or standing quietly—and begin pecking the feathers on the underpart of her body. Such behavior is usually followed by snuggling, in which the chick rubs and pushes its head up into the hen's feathers. These behaviors appear to stimulate the hen to sit. The sitting hen makes sounds and movements that then attract the other chicks to be brooded.

The hen initiates brooding on many occasions, but she is less likely than normal to sit and initiate brooding in the observation cage when she is slightly disturbed by the presence of an audience. Under these circumstances, the chicks become cool and frequently show the pecking and snuggling behaviors described above.

In light of these observations, one can interpret the behavior of Wasserman's chicks toward the lighted key as part of normal heat-seeking behavior redirected toward a new stimulus. Thus, it seems premature to postulate any new determinants of the form and direction of the conditioned responses in conditioning studies.

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Hogan describes the behavior of young chicks in the presence of a broody hen as frequently involving approach, pecking, and snuggling behaviors. He further suggests that these observations bear upon the conclusions I drew from an investigation employing heat stimulation (*1*). In that study, chicks in a cooled chamber were irradiated from an overhead heat lamp at random intervals. Experimental subjects had each heat presentation signaled by the lighting of a small key, whereas control subjects received random presentations of key-light and heat stimuli. The results were that only the experimental subjects learned to approach and to peck at or snuggle with the lighted key. These conditioned responses arose despite the fact that diffuse warming of the chick's chamber elicited reduced locomotion, extension of the wings, twittering, and eye closure.

I interpreted these results as supportive of the view that, in addition to the behavior-eliciting properties of unconditioned stimuli, such physical characteristics of conditioned stimuli as their accessibility and localizability may participate in determining the form and direction of responses conditioned with Pavlov's procedure (*2*). This proposal represents an elaboration of the principle of stimulus substitution, which holds that the topography of the conditioned response ought to be a "replica" of the unconditioned response (*3*). The conditioned responses that I observed seemed to be more directly related to the physical properties of the small lighted key than to the increase in ambient temperature. Woodruff and Williams (*4*) have also made observations that call for a modification of the stimulus-substitution hypothesis. These researchers paired the lighting of a small key with the delivery of water directly into the mandibles of thirsty pigeons. Although the introduction of water into the bill did not elicit directed skeletal behavior, but rather swallowing, subjects learned to approach and contact the lighted key. Both of these studies clearly indicate that directed skeletal behaviors may be conditioned to localized conditioned stimuli even when they are not elicited by the reinforcing stimulus.

Hogan's comments do not distinguish between the behaviors that precede the reinforcing stimulus and those that follow its reception. At issue is how to explain the control of appetitive "heat-seeking" behaviors as distinct from the factors that control consummatory "heat-elicited" behaviors (*5*). With this distinction in mind, it may be that Hogan's observations do not refute my earlier conclusions—they may even support them. If we assume that the broody hen serves the dual functions of a localized visual stimulus and a heat source, and that the sight and warmth of the hen have been repeatedly paired during the chick's first week of life, then approach and contact may come to be controlled by the former stimulus property while body lowering and wing extension are evoked by the latter (*6*). Here, laboratory findings permit us to unconfound and elucidate naturalistic observations.

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2. When the lighting of a nickel-sized key is repeatedly followed by food, hungry pigeons learn to approach and peck the lighted key [P. L. Brown and H. M. Jenkins, *J. Exp. Anal. Behav.* **11**, 1 (1968)]. However, when auditory stimuli are paired with grain, subjects do not frequently engage in pecking at the speaker or any other environmental features [J. Bilbrey and S. Winokur, *ibid.* **20**, 323 (1973); B. Schwartz, *ibid.*, p. 17; G. W. Farthing, *Psychonom. Sci.* **23**, 343 (1971); E. A. Wasserman, thesis, Indiana University (1972)]. Presumably, the different physical characteristics of punctate visual stimuli compared to diffuse auditory stimuli are responsible for the failure of appreciable pecking to develop with auditory signals. Further evidence and discussion bearing on this issue have been presented D. Bindra [*Psychol. Rev.* **81**, 199 (1974)]; E. A. Wasserman [*Anim. Learn. Behav.* **1**, 198 (1973)]; E. A. Wasserman, S. R. Franklin, E. Hearst [*J. Comp. Physiol. Psychol.* **86**, 616 (1974)]; E. A. Wasserman and S. B. McCracken [*J. Exp. Anal. Behav.* **22**, 39 (1974)].
3. A discussion of various interpretations of the stimulus-substitution notion was presented by H. M. Jenkins and B. R. Moore [*J. Exp. Anal. Behav.* **20**, 163 (1973)].
4. G. Woodruff and D. R. Williams, paper presented at the 1974 meeting of the Eastern Psychological Association, Philadelphia, Pa.
5. The distinction between appetitive and consummatory responses was discussed W. Craig [*Biol. Bull.* **34**, 91 (1918)].
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## Viscosity of Cellular Protoplasm: What Do Spin Probes Tell Us?

Keith and Snipes (*1*) recently studied the electron spin resonance (ESR) line shape of a spin probe (tempone) dissolved in cellular protoplasm. By comparing the line shapes with those from the spin probe in glycerol-water mixtures of known viscosity, they concluded that protoplasmic viscosity in some cells is many times that of pure water.

According to the Stokes-Einstein hydrodynamic approach, viscosity should